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REVIEW

Mechanisms of Tumor Promotion by Reactive Oxygen Species

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Abstract—This review analyzes the available information concerning mechanisms of non-genotoxic action of reactive oxygen species (ROS) during tumor promotion and pathways of their generation under the influence of chemical compounds. Special attention is given to the ability of ROS to induce pseudohypoxia through inhibition of prolyl oxidase, which is an oxygen sensor in the cell. Functions of HIF-1α as a main contributor to the ROS-induced promotion are analyzed. Data suggest that an unregulated high level of HIF-1α in the cell could induce the development of tumors. Hypothetical possibilities of ROS production under the influence of different environmental pollutants, which are promoters of tumorigenesis, include functioning of cytochrome *P450* during oxidation of substrates, functioning of the mitochondrial respiratory chain, and action of peroxisome proliferators.

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The initial stages of carcinogenesis under the influence of chemical substances can be divided into at least two stages: initiation and promotion. Compounds possessing both initiating and promoting activity are known ("complete" carcinogens), as well as substances possessing only promoting properties. These stages are fundamentally different: the initiation stage is caused by hereditary changes in the cell genome under the influence of electrophilic genotoxic metabolites of carcinogens, whereas the promotion stage is mediated by a non-genotoxic (epigenomic) mechanism. The promotion stage of carcinogenesis creates conditions for the preferential growth of the produced initiated cells [1] to the state when the tumor size becomes sufficient for its autonomous functioning without support from outside by the promoter. Promotion requires the following conditions: stimulation of proliferation, inhibition of intercellular interactions allowing the initiated cells to escape the regulatory influence of the surrounding normal cells, and inhibition of apoptosis. As differentiated from the initiation stage, which seems to be short-term and irreversible, the non-genotoxic action of promoters should be sufficiently long-term and on its termination no tumor arises. While the mechanism of the initiation stage is rather well studied and structure of the "final" carcinogen is known in many cases, the promotion stage much less well studied. It is unknown if there is a common promotion mechanism or if this stage of carcinogenesis proceeds through several pathways depending on the agent structure and cell type influenced by the promoter.

A large group of tumor promoters consists of such environmental pollutants as chlorinated hydrocarbons, barbiturates, and clofibrate-type peroxisome proliferators. Environmental pollutants also include components of exhaust gases of internal combustion engines – polycyclic aromatic hydrocarbons (PAH). PAHs are "complete" carcinogens, i.e. their structure ensures their ability to catalyze both the initiation and promotion stages and induce carcinogenesis without additional influences. All these compounds can act as both substrates and inducers of cytochrome *P450* isoforms. The tumor-promoting action of these substances is now thought to be due to either functioning of cytochrome *P450* isoforms or activation of receptors involved in transmission of the inducing signal. Three types of receptors are known that act as sensors of xenobiotic entrance into the cell: aryl hydrocarbon receptor (Ah receptor), constitutive active androstane receptor (CAR), and peroxisome proliferator receptor (PPAR). Mice with knockout of the receptor interacting with a corresponding xenobiotic were shown to lose their sensitivity to the promoting effect of this compound [2, 3].

It is possible that the activated receptors can influence the transmission of regulatory signals (e.g. mitotic or antiapoptotic ones) and thus cause promotion. The effect of promoters can also be indirect, via formation of a compound acting as a direct promoter.

The promoter-induced production of reactive oxygen species (ROS) in the cell is considered to be a possible mechanism of promotion. ROS are natural products of cell vital activity, and many researchers consider them as secondary messengers in the transmission of regulatory signals. Note that in physiological concentrations ROS can reversibly change the level of reduced thiol groups in protein molecules and thus change their functional activity. It is reasonable to think that ROS are involved in the transmission of mitotic signal [4-6]. ROS are necessary for signal transmission on activation of the JNK-dependent pathway. In the inactive state JNK is complexed with *p*-glutathione S-transferase and cannot be phosphorylated by the previous member of the mitotic cascade, the protein MKK 4/7. To activate JNK, ROS must oxidize SH-groups of *p*-glutathione S-transferase with the resulting detachment of the latter from JNK and the acquisition by JNK of the ability for signal transmission [7-9]. Studies on the cell cycle revealed that transmission of the proliferation signal was associated with an increase in production of ROS at the late G1-phase along with an increase in mitochondrial mass, and that the addition of antioxidants prevented synthesis of cyclin A responsible for the cell entrance into the S-phase and, as a result, prevents this stage of proliferation [4]. Transmission of the mitotic signal from growth factors by ROS is associated with oxidation of thiol groups of tyrosine phosphatases, which results in their reversible inactivation and facilitates proliferation [10]. Thus, ROS act as proliferation stimulants similarly to proto-oncogenes. Constant disorders in functioning of oncogenes associated with their increased expression or disorders in the regulation and the steadily increased level of ROS can lead to the same results – an unregulated proliferation and finally development of a tumor. Induction of promotion caused by the constant inactivating of tyrosine phosphatases was shown using phosphatase inhibitors such as okadaic acid, nodularin, and other tumor promoters [11].

The ability of ROS to stimulate the promotion stage of carcinogenesis has been shown in some models. Such source of ROS as benzoyl peroxide is a promoter [12]. The hydroperoxide of linoleic acid has tumor-promoting properties [13]. Injection to animals with skin carcinogenesis caused by 7,12-dimethylbenz/a/anthracene (DMBA) of the photosensitizer dihematoporphyrin and a subsequent UV-irradiation of the skin induced production of ROS and had a promoting effect. In the absence of irradiation, dihematoporphyrin did not display the promoting action [14]. The ROS generation by Kupffer cells in liver tumor was responsible for the promotion stage of carcinogenesis induced by diethylnitrosoamine [15] or peroxisome proliferators [16]. Inflammation is generally thought to be a factor increasing the risk of development of malignant tumor [17].

Production of ROS increases under the influence of various promoters of carcinogenesis. Thus, the promoting activity of phorbol esters correlated with their ability to induce the oxygen burst in macrophages, and injection of antioxidants prevented the promotion [18, 19]. Peroxisome proliferators promoting liver tumors increased production of H_2O_2 during carcinogenesis [20]. Chlorinated hydrocarbons with tumor-promoting features increased production of ROS in cell culture [21]. Promoters of the barbiturate class also induced oxygen stress in both *in vivo* and *in vitro* experiments [22].

Production of ROS and of the ROS-induced oxidative stress can be recorded by appearance within the cellular DNA of the guanine oxidation product 8-hydroxydeoxyguanidine. This compound can be detected in cell culture and also in the liver in the presence of different inducers of cytochrome *P450* [23, 24]. Injection *in vivo* of an effective tumor promoter of chlorinated dioxins induced oxidative stress manifesting itself by a 4-5-fold increase in lipid peroxidation, a decrease in the fluidity of mitochondrial and microsomal membranes, and an increase in the level of calcium ions in mitochondria and microsomes [25]. The damage to DNA under the influence of substances stimulating production of ROS seems to indicate a mutagenic character of ROS action resulting in events similar to those induced by carcinogens. Nevertheless, this is unlikely to be the main factor determining the tumor-promoting action of ROS. The promoting action must be rather long-term and uninterrupted, and withdrawal of the agent prevents the development of tumor, which indicates the reversibility of the promoting effect and suggest an epigenetic action of ROS.

Note that the effect of ROS on cell functions depends on their concentration. Physiological concentrations of ROS are involved in the regulation of cellular processes. At higher concentrations (or at physiological but constant unregulated levels) ROS stimulate proliferation and induce a pseudohypoxic state; the further increase in the ROS concentration causes cell death through apoptosis, irreversible disorders in the structure of macromolecules, and then the massive death of cells through necrosis (Fig. 1).

It seems that the stimulation of proliferation through inhibition of phosphatases is not the only tumor-promoting action of ROS: other disorders in cell functioning associated with tumor promotion are also observed – intercellular interactions are changed, cell migration is stimulated, and apoptosis is inhibited [6]. However, there is no definite answer to the question of what the pathway inducing these changes by ROS is. The ability of ROS to induce pseudohypoxia seems to be the most interesting possible mechanism of their promoting action.

The switching of cells from normoxia to hypoxia is regulated by the protein HIF-1α. In the normoxia state this protein is oxidized under the influence of prolyl oxidase (PO) at prolines in positions 402 and 564 [26]. PO can be considered to be a sensor of the oxygen level in the cell. HIF-1α with oxidized prolines interacts with a ubiq-

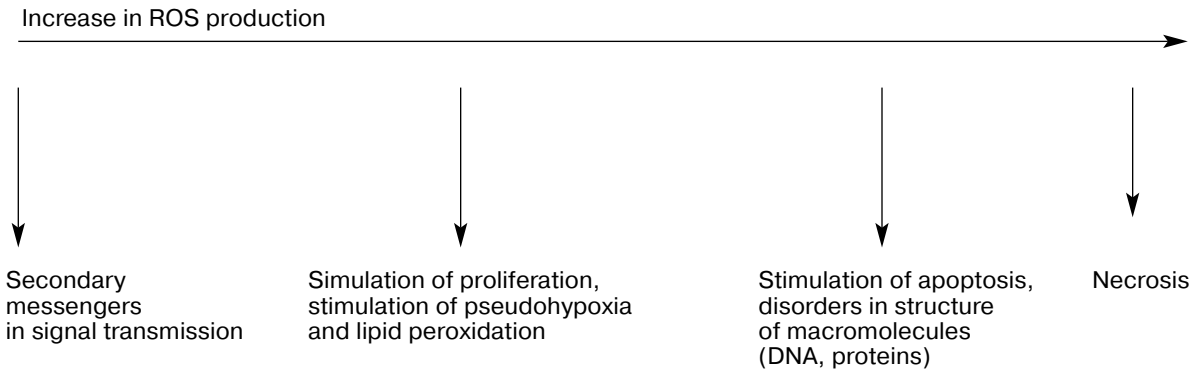

Fig. 1. Effects of reactive oxygen species (ROS) on cell functions depending of the level of ROS production.

uitin ligase, the protein VHL [6, 27], and is destroyed. At insufficient level of oxygen, PO does not oxidize HIF-1α, the protein VHL does not interact with HIF-1α, and the amount of HIF-1α increases. HIF-1α interacts with the protein ARNT that is characterized by constant intracellular level, and the complex HIF-1α–ARNT starts functioning as a transcription factor stimulating expression of more than 200 genes [28] (Fig. 2) that encode proteins involved in glycolysis, proliferation, and immortalization; apoptosis is also inhibited [29]. The regulation of other cellular processes is also changed. The PO-catalyzed oxidation of HIF-1α at prolines is coupled with co-oxidation of 2-oxoglutarate to succinate [26, 30]. Inhibition of 2-oxoglutarate oxidation is associated with inhibition of HIF-1α oxidation of prolines. The product of 2-oxoglutarate oxidation, succinate, inhibits oxidation of this compound [30, 31]. Another mitochondrial product, fumarate, also inhibits PO [29, 30]. Therefore, accumulation in cells of succinate or fumarate due to inhibition of succinate dehydrogenase or fumarase, respectively, results in an increase in the amount of protein HIF-1α and in pseudohypoxia. The constant generation of ROS above the physiological level can cause pseudohypoxia due to direct inactivation of PO. ROS oxidize components of the enzyme active center, which contains bivalent iron, and also ascorbate, which supports iron in the reduced state [30, 32]. The ability of ROS to induce pseudohypoxia is confirmed by the observation that addition of hydrogen peroxide to cells increased the level of the protein HIF-1α [33, 34]. Cyclic oxidation of 2,3-dimetoxy-1,4-naphthoquinone inside cells was associated with production of superoxide, and its injection into various cells caused a concentration-dependent increase in the level of ROS and accumulation of HIF-1α [35].

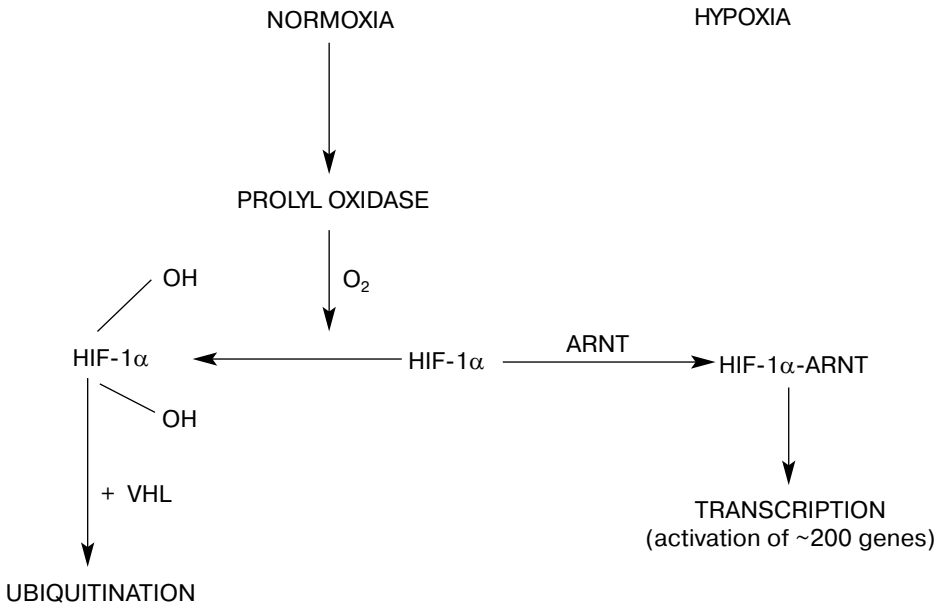

Fig. 2. Regulation of HIF-1α level in cells under conditions of normoxia and hypoxia. See text for details.

Treatment of cells with interleukin-1 induced in them the generation of ROS and accumulation of HIF-1α [36]. ROS were produced in the mitochondrial respiratory chain, because inhibitors of the mitochondrial chain of electron transfer prevented accumulation of HIF-1α [36]. ROS produced by stomach epithelial cells infected with *Helicobacter pylori* also induced pseudohypoxia [37]. The existence of hereditary tumors associated with an increased level of HIF-1α under conditions of normoxia also suggests the involvement of HIF-1α in carcinogenesis and tumor progression. Hereditary inactivating mutations in the *VHL* gene resulting in accumulation of HIF-1α promote appearance of such tumors as retina angiomas, brain angiomas, and light-cell kidney carcinomas [38]. Hereditary inactivating mutations in the genes of succinate dehydrogenase or fumarase resulting in the accumulation in cells of succinate and fumarate inhibiting PO activity are likely to be the cause of hereditary paragangliomas, pheochromocytomas, and kidney tumors [39, 40]. Therefore, the genes encoding these compounds were assigned to suppression genes [26, 30, 38]. In a recent review [40] the author analyzed mutations in mitochondrial DNA in tumors and concluded that the majority of these multiple mutations were not associated with arising of tumors, except mutations in germ cells in the genes encoding succinate dehydrogenase and fumarase responsible for hereditary predisposition for tumors.

The genes encoding succinate dehydrogenase and fumarase are very sensitive to ROS during chemical carcinogenesis. Thus, injection into A549 cells of low doses of carcinogenic nickel salts caused oxidative stress and disorders in functioning of these genes [41]. Activities of different enzymes of respiration were studied histochemically in kidney carcinogenesis induced by injection of 1,2-dimethylhydrazine, and a significant decrease was found in the succinate dehydrogenase activity [42]. In mouse liver mitochondria the succinate dehydrogenase activity was decreased and lipid peroxidation was increased during DMBA-induced carcinogenesis [43].

Cells subjected to hypoxia displayed increased proliferation [44, 45], stoppage of differentiation [46, 47], an increased ability for migration and invasiveness [48, 49], and resistance to apoptosis [50, 51]. In the state of hypoxia energy provision of cells is switched from respiration to glycolysis. Thus, hypoxia is a powerful factor changing the overall cellular homeostasis.

EFFECTS CAUSED BY ACTIVATION OF HIF-1α

Glycolysis. Under conditions of oxygen insufficiency the cell has to use glycolysis, which is less efficient for energy provision than respiration. HIF-1α transcribes the gene of glucose transporter and genes whose products are necessary for glycolysis, including the gene of hexokinase II [52], which is involved in the antiapoptotic effect realized through an increase in the expression of HIF-1α. The gene of pyruvate dehydrogenase kinase is also activated [53], and this kinase phosphorylates pyruvate dehydrogenase, which converts pyruvate into acetyl coenzyme A and triggers the tricarboxylic acid cycle. Phosphorylation of pyruvate dehydrogenase is associated with its inactivation. Thus, in addition to activation of glycolysis, functioning of HIF-1α is associated with inhibition of the mitochondrial respiratory chain. Switching off respiration under conditions of hypoxia and oxygen insufficiency seems to be due to generation by the mitochondrial respiratory chain of large amount of ROS that can be toxic for the cell.

Immortalization. The acquisition by target cells of the ability for unlimited division (immortalization) is one of the most important factors of carcinogenesis. Limited number of cell divisions and limited lifetime are determined by shortening of DNA ends (telomeres) at every division. An immortalization mechanism necessary for the unlimited growth of tumor cells and recovery of the length of telomeres is activation of telomerases, which is not expressed in the majority of adult cells. In particular, the gene encoding telomerase is transcribed on HIF-1α activation [54, 57].

Stoppage of differentiation. Tumor cells are characterized by a decrease or complete absence of differentiation parameters characterizing the organ of tumor origin. This phenomenon is associated with the mechanism of tumorigenesis. According to the most accepted modern viewpoint, the origin of tumorigenesis is a transformed stem cell, which, similarly to the normal stem cell, produces during the division a similar non-differentiated cell or a cell capable only of limited differentiation. The ability for tumorigenesis is supported by a small population of tumor stem cells [58, 59]. Therefore, retention of these cells in the initial "stem" state is necessary for tumor growth. It is also possible that the cell is transformed at a certain stage of differentiation, i.e. with an unfinished differentiation or with some differentiation parameters lost during tumor growth. Thus, in all the above-mentioned cases tumorigenesis is started by a cell that is less differentiated than the surrounding "mature" cells. In particular, hypoxia mediated through HIF-1α supports the polypotent state of stem cells. This is confirmed by the hypoxic state of cells during early stages of embryogenesis [60, 61]. In the bone marrow stem cells are located in regions with low level of oxygen [84]. The stoppage of cells during differentiation is also regulated by transcription factor HIF-1α. Under the influence of hypoxia, preadipocytes are shown to reversibly maintain the non-differentiated state, and the authors [47] believe that just HIF-1α is a factor supporting the cells in the non-differentiated state under conditions of hypoxia.

Expression of the gene *Oct-4* is also important for supporting the polypotent state of stem cells. Expression

of this gene is not recorded in the cells of an adult organism but is observed in embryonic and stem cells. Expression of the *Oct-4* gene is dramatically increased on functioning of transcription factor HIF-1α [63].

Another mechanism of supporting stem cells in the polypotent state and changing the differentiation status of the cells is associated with activation of the protein Notch by protein HIF-1α [64, 65]. Notch is a transmembrane receptor with an intracellular region (ISL) detached upon interaction of the extracellular part with a ligand. ISL is transported into the nucleus where it activates the transcription factor CSL (CBF1 in humans) [66, 67]. And depending on the cell type, this results in inhibition or stimulation of differentiation. Thus, Notch supports intestinal stem cells in the polypotent state and stimulates the terminal differentiation of skin cells [67]. Disorders in the *Notch*-dependent pathway of signal transmission can result in tumorigenesis, in particular, in T-cellular neoplasia [67]. There is no definite answer to the question how *Notch* activation occurs under conditions of hypoxia. Some data show that HIF-1α induces the *Notch* gene and its ligands [61], whereas other data suggest that HIF-1α interacts with ISL and increases the transcriptional activity [66, 66a]. But possibly the *Notch* gene can be activated through both mechanisms.

Apoptosis. Inhibition of apoptosis is necessary for tumor development in the promotion stage because an increase during carcinogenesis of the cell quantity in the organ above a programmed number is accompanied by triggering the cell suicide mechanism to maintain constant cell number in the organ. There are reports about both inhibition [69] and stimulation of apoptosis under conditions of oxidative stress [70]. But it should be noted that the majority of studies that detected activation of apoptosis under conditions of hypoxia were performed in the state of "deep" hypoxia, at the oxygen level close to anoxia, i.e. in the situation observed in a rapidly growing tumor. Data presented in the work [71] show that in the state of anoxia the HIF-1α activity is inhibited through the p53-dependent pathway, whereas no such inhibition occurs under conditions of hypoxia. In the state of "pseudohypoxia" during carcinogenesis the oxygen concentration is sufficient and the effects are associated only with HIF-1α functioning. HIF-1α increases transcription of genes encoding synthesis of antiapoptotic proteins. These proteins switch on some antiapoptotic mechanisms. One of these mechanisms is associated with an increased transcription of survivin, an antiapoptotic protein of the IAP family [72, 73]. The mechanism of the antiapoptotic action of survivin is not quite clear, but similarly to other proteins of the IAP family it is supposed to block activities of caspases, first of all caspase 9 [72, 74]. Survivin is expressed in embryonic tissues and is not detected in differentiated cells of an adult organism. Expression of survivin is found in differently located tumors, and the use of the expression of this protein as a possible marker of tumor growth is discussed [75]. Expression of survivin appears in the target organ during early stages of DMBA-induced carcinogenesis, whereas such expression in the control animals is absent [76]. The cells can also withstand apoptosis through expression of hexokinase II. In addition to phosphorylation of glucose, this enzyme blocks mitochondrial voltage-dependent anionic channels (VDAC) [77-79], which allow cytochrome *c* to enter the cytoplasm during apoptosis [78, 81] and make apoptosis irreversible. The inhibition mechanism is not quite clear, but some data suggest that apoptosis can be inhibited due to phosphorylation of VDAC by protein kinase C, which is activated by HIF-1α-regulated hexokinase [80]. Other studies indicate that hexokinase interacts directly with VDAC and changes the conformation of the pore produced by this protein, and this change prevents the exit of cytochrome *c* through this pore and inhibits apoptosis [77, 78, 81].

The antiapoptotic action of HIF-1α is also mediated by an increase in expression of the protein nucleophosmin [82]. This protein prevents activation of protein p53 due to inhibition of its phosphorylation by serine 15 [82-84]. HIF-1α also increases expression of the *Mcl-1* gene (myeloid cellular factor 1), which synthesizes an antiapoptotic protein of the bcl2 family [85]. The protein Mcl-1 inhibits the activity of caspase 3 and protects cells against toxic action of chemical compounds.

DISTURBANCE OF INTERCELLULAR INTERACTIONS

Intercellular interactions are an important factor for the regulation of organs. During carcinogenesis and tumor growth these interactions are affected, connections between the tumor cells are weaker, and therefore they can separate from the tumor bulk and be transported along the blood channel initiating metastases. Cell morphology is also changed, and the so-called epithelial–mesenchymal transition (EMT) is observed: the epithelial cells acquire a fibroblast-like structure and mesenchymal markers, such as fibronectin, appear [86]. The fibroblast-like structure provides for greater mobility and invasiveness of the cells. Intercellular adhesive interactions are regulated by cadherins. Cadherins are calcium-dependent transmembrane proteins responsible for adhesive contacts between neighboring cells. During EMT the expression of cadherins decreases. Hypoxia stimulates EMT [87, 88] and decreases the expression of cadherins. Inactivation of the gene *VHL* resulting in an increase in the HIF-1α level also induces EMT in cell culture [89]. These data show that the hypoxia-caused EMT is realized due to functioning of HIF-1α. It seems that on accumulation in the cell of HIF-1α, EMT can be induced through some mechanisms. The fall in the expression of cadherins is associated with an increase in the expression

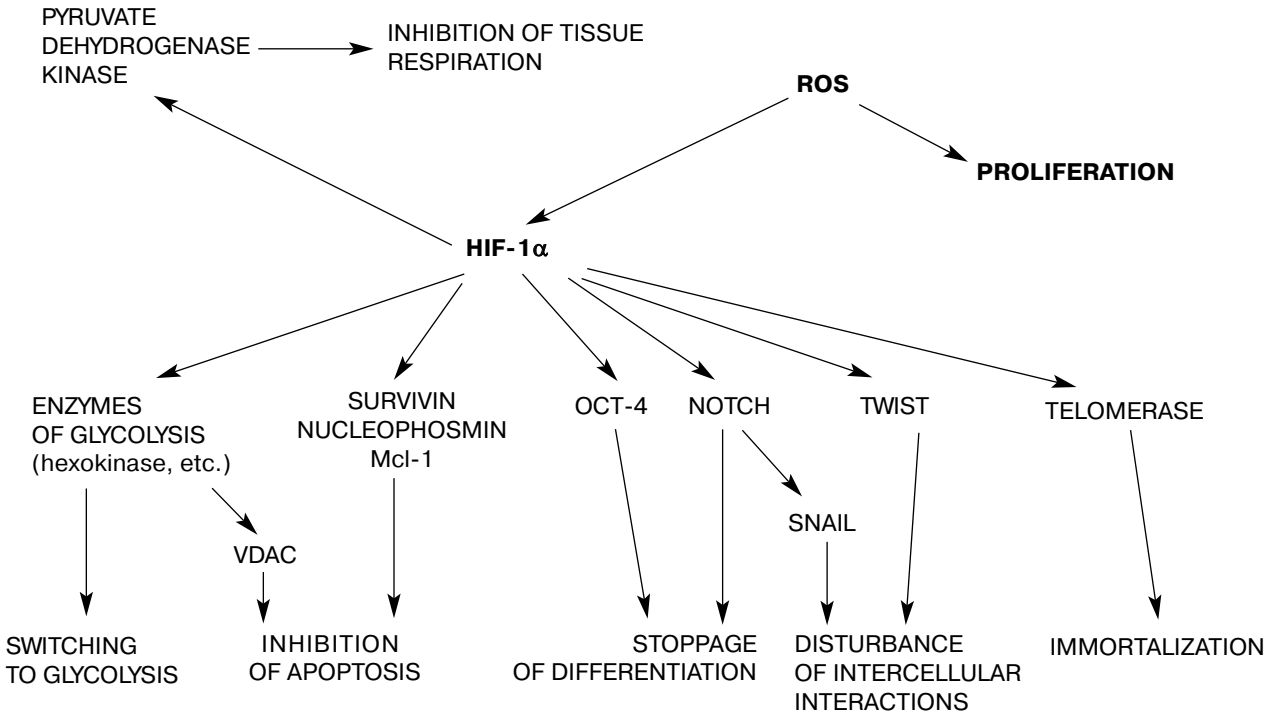

Fig. 3. Influences of reactive oxygen species (ROS) on promotion-associated cell functions.

of their repressor – proteins of the SNAIL family [87, 88]. The mechanism of the increase in expression of the SNAIL family proteins upon HIF-1α activation is not clear. On one hand, the above-mentioned receptor Notch activated by hypoxia is shown to increase the expression of SNAIL [87, 90]. Moreover, HIF-1α induces an increased expression of lysyl oxidase, which stabilizes SNAIL [91] and increases its inhibiting effect on the expression of cadherins. Another protein, TWIST, which is also an inhibitor of cadherins [92-94], has a HIF-1α-binding element in the promoting region of the gene [95]. In the same works it is also shown that injection of TWIST siRNA into cells with hyper-expressed HIF-1α decreases EMT and the ability of cells to metastasize. The authors think that the HIF-1α-dependent expression of TWIST is the main factor of EMT under conditions of hypoxia. (The overall scheme of ROS action on cell functions is presented in Fig. 3.)

PRODUCTION OF ROS UNDER THE INFLUENCE OF CHEMICAL CARCINOGENS

The mechanisms of ROS generation under the influence of carcinogenesis promoters depend on their structure. As "primary" ROS in cells, superoxide anion (which dismutates to hydrogen peroxide) as well as hydrogen peroxide can be formed. Superoxide anion is generated during oxidation of substances easily donating electrons, such as nicotinamide and flavones. Superoxide is also produced as a byproduct in the oxidative cycle of carcinogen oxidation on cytochrome *P450*. Hydrogen peroxide is produced from superoxide under the influence of superoxide dismutase (SOD) or in some enzymatic reactions directly on substrate oxidation, as in the case of oxidation of fatty acids.

Generation of ROS during functioning of cytochrome *P450* isoforms. Many promoters of carcinogenesis are simultaneously substrates and inducers of cytochrome *P450* isoforms. The induction by a given compound significantly increases the level of the corresponding isoform of cytochrome *P450*. ROS can be generated on cytochrome *P450* during the oxidation cycle [1, 96-98]. Generation of ROS is an accessory reaction during the substrate oxidation as a result of disintegration of the triple complex of enzyme–substrate–superoxide with production of free superoxide, which is converted into hydrogen peroxide under the influence of superoxide dismutase (SOD). The induction is associated with an increase in the level of cytochrome *P450*, thus, the amount of produced ROS also increases. Therefore, for production of ROS a compound must be not only a substrate, but also an inducer of cytochrome *P450* isoforms.

Isoforms of cytochrome *P450* seem to differ in their ability to stimulate production of ROS during oxidation of xenobiotics. The production of ROS is most effectively catalyzed by the cytochrome isoform *P4502B1* [99], which in its turn is induced by phenobarbital-type pro-

moters of carcinogenesis and also by some chlorinated hydrocarbons.

During normal cell functioning, the greatest amount of ROS is produced in the mitochondrial respiratory chain. It is thought that from 1 to 4% of oxygen consumed by the mitochondrial respiratory chain is converted into ROS [100]. Interest in studies on the role of mitochondria in carcinogenesis has not disappeared since Warburg's time, i.e. from the 1920s up to the mid-1970s when oncogenes and later suppression genes were shown to exist. However, in recent years interest increased in studies of the role of mitochondria in carcinogenesis. On one hand, arising of tumors not always can be explained only by disorders in the regulation of expression or in the structure of oncogenes and suppression genes. Moreover, some earlier experimental data could not be explained by existing hypotheses. Thus, the cytoplasm of tumor cells retained the tumor phenotype on fusion with nuclei of normal cells [101-103]. This suggested the responsibility of cytoplasmic factors for tumor phenotype. Mitochondria are the cytoplasmic structures most suitable as a "carcinogenic factor". Mitochondrial stress caused by different situations (exhaustion of mitochondrial DNA, injection of mitochondrial toxins similar to respiration–phosphorylation uncouplers) induced progression of the tumor phenotype manifested by appearance of invasiveness in non-invasive cells and also by resistance to apoptosis [104-106]. An increased generation of ROS under the influence of carcinogens can also be a mechanism of disorders in mitochondrial functioning resulting in mitochondrial stress.

Experiments changing the ROS level in mitochondria confirm that the excess production of ROS can be a carcinogenic factor. SOD, which catalyzes the conversion of superoxide into hydrogen peroxide, is a factor of antioxidant defense in the cell. SOD is present in the cell as three isoforms located in different cell compartments. SOD1 and SOD2 are located in mitochondria and convert superoxide ion produced in the respiratory chain into hydrogen peroxide. Manganese-dependent SOD (SOD2) is located in the mitochondrial matrix. Mice with *SOD2* gene knockout are nonviable and dye immediately after birth [112]. Heterozygous mice have oxidative disorders in DNA and an increased level of malignant neoplasms [112]. Cu-Zn-dependent SOD1 is located in the mitochondrial intermembrane space. Mice with *SOD1* gene knockout have shortened lifespan associated with development of liver tumors [113]. However, the number of skin tumors induced by DMBA and subsequent treatment with phorbol ester was lower in transgenic mice with hyper-expressed *SOD2* than in "wild" animals [114]. In the transgenic animals there were no disorders in the structure of mitochondria, and disorders specific for oxidative stress and observed in the skin of "wild" mice were less pronounced [115]. These data indicate that ROS produced in mitochondria can cause development of tumors under conditions of chemical carcinogenesis. SOD3 is located in the extracellular space and protects components of the intercellular space against ROS produced during inflammation.

Some carcinogens induce an increased production of ROS in the mitochondrial respiratory chain, and this can contribute to their promoting effect.

Phorbol esters are widely used as tumor promoters. These compounds are structural analogs of diacylglycerols, which are natural activators of protein kinase C. The ability to activate protein kinase C is thought to be the first stage of the promoting action of phorbol esters. But how activation of protein kinase C results in promotion was unclear. Meanwhile antioxidants were shown to inhibit the promoting effect of phorbol esters in cell culture [18, 19], and this suggested a ROS-dependent action of these promoters. Analysis of the ROS generation pathways under the influence of phorbol esters revealed that they were produced in mitochondria [120]. The most efficient tumor promoter of the phorbol ester family, phorbol-12-miristate-13-acetate, was recently shown to cause translocation of protein kinase C into mitochondria of various cells. The translocation was accompanied by an increase in the protein kinase C activity, changes in the morphology of mitochondria, a decrease in the transmembrane potential, and decreases in complex 1 activity in the mitochondrial respiratory chain and in the activity of pyruvate dehydrogenase. Generation of ROS in the mitochondria was increased [121].

Some data suggest that various tumor promoters of Ah receptor-activating substances, which are inducers of the cytochrome *P450* family 1 isoforms, can cause oxidative burst in mitochondria.

In the early 1990s 2,3,7,8-tetrachlorodibenzoparadioxin (TCDD), which is a promoter of hepatocarcinogenesis, an effective inducer of cytochrome *P450* isoforms, and a ligand of the Ah receptor was shown to increase in mitochondria and in the microsomal fraction the level of ROS that was recorded by luminescence, reduction of cytochrome *c* [107], and a fourfold increase in lipid peroxidation in the mitochondrial membrane [108, 109]. A similar effect was also caused by injection into animals of tobacco gum containing PAH and other ligands of the Ah receptor [110]. The increase in the generation of H_2O_2 in the liver mitochondria of animals treated with TCDD was also confirmed by a ~80% decrease in the activity of aconitase, which is very sensitive to H_2O_2 [111].

In the mitochondrial membrane, cytochrome *P450* isoforms *1A1* and *1A2* are expressed similarly to those expressed in the endoplasmic reticulum. Under certain conditions these isoforms can generate ROS in the presence of some promoters that are inducers of these enzymes. Cytochrome *P450* isoforms *1A1* and *1A2* are induced due to functioning of the Ah receptor. TCDD, which is an inducer of cytochrome *P450* isoforms *1A1*

and *1A2* and also a promoter of carcinogenesis, increased the level of cytochrome *P450* isoforms in both the endoplasmic reticulum and mitochondria [116]. But while the induced level of cytochrome *P450* was retained in microsomes for one week after the injection of TCDD, the induced level of cytochrome *P450* in mitochondria was retained during eight weeks. The authors supposed that the TCDD-caused oxidative stress should be mediated through functioning of the induced mitochondrial isoforms of cytochrome *P450* resulting in disorders of mitochondrial functions. This hypothesis was tested in work [117], and it was shown that in animals with knockout of the genes of cytochromes *P4501A1* or *P4501A2*, TCDD caused an insignificant decrease in the ROS generation in the liver mitochondria. However, in animals with knockout of the Ah receptor the constitutive level of ROS generation in mitochondria was 20% of the level observed in the "wild" animals. In animals with knockout of the Ah receptor, injection of TCDD did not increase the ROS generation. Thus, carcinogenesis promoters, which are simultaneously ligands of the Ah receptor and inducers of cytochrome *P450* isoforms, can cause oxidative stress in mitochondria with greater probability (due to functioning of the activated Ah receptor) than inducers of cytochrome *P450* isoforms. The promotion under the influence of the Ah receptor ligands is realized mainly due to activation of the Ah receptor and not to ROS production by cytochrome *P450*, and this is confirmed by promotion of hepatomas [118] and stomach tumors [119] in transgenic animals with constitutively active Ah receptor without injection of ligands of this receptor.

Using an inhibitor of succinate dehydrogenase, thenoyltrifluoroacetate, it was shown [117] that under the influence of TCDD, ROS were produced in the mitochondrial respiratory chain in the succinate-dependent region of electron transfer in complex 2. Injection of TCDD also caused in animals a decrease in ATP synthesis. However, the respiratory control in liver mitochondria of the TCDD-injected animals did not change, but consumption of O_2 increased approximately twofold in both state 3 and 4 as compared to liver mitochondria of the control animals. Increases were also observed in the activity of glutathione reductase and in the level of reduced glutathione in the liver mitochondria of the TCDD-treated animals, but the level of oxidized glutathione was unchanged. The authors think that this also suggests oxidative burst in mitochondria [110, 111, 117]. A more careful study on the influence of TCDD on mitochondrial functions revealed a decrease in the level of ubiquinone and an increase in the activity of ubiquinone oxidase a week after the TCDD injection. This was associated with a decrease in ATP synthesis and in the ATP/O_2 ratio without changes in the respiratory control value. In a more exotic model, spermatozoa, TCDD increased the level of ROS in mitochondria, decreasing the membrane potential [122]. This effect also depended on the Ah receptor because in mice with knockout of this receptor neither ROS generation nor a decrease in the membrane potential was observed. ROS generation in the respiratory chain of mouse mitochondria increased during the period of 7-28 days after injection of TCDD at the level of succinate dehydrogenase [123], and the number of structural damages in mitochondrial DNA was twofold higher than in nuclear DNA, possibly because of the action of ROS. In particular, the increased generation of ROS in mitochondria under the influence of the Ah receptor ligands was indicated by inhibition by the activated Ah receptor of activity of complex IV (cytochrome oxidase) in the respiratory chain of mitochondria along with the increase in activities of complexes II and III. This results in increased production of ROS in complexes II and III. Because the activated Ah receptor is a transcription agent, TCDD is supposed to induce expression of an unknown gene encoding a protein activating electron transfer at the level of complexes II and III and inhibiting electron transfer at the level of cytochrome oxidase [117].

We think that the increased generation of ROS in mitochondria under the influence of promoters and the carcinogenicity of ROS produced in mitochondria are proved convincingly. Nevertheless, there is no unambiguous idea about the pathway of carcinogen-induced increase in the ROS generation in mitochondria and about similarity of the mechanism of ROS production under the influence of various substances.

Peroxisome proliferators can be another source of ROS generation under the influence of carcinogenesis promoters because just in these organelles β-oxidation of fatty acids is accompanied by production of hydrogen peroxide. In addition to the increase in number of peroxisomes and respectively in the amount of enzymes oxidizing fatty acids, peroxisome proliferators induce synthesis of cytochrome *P450* isoforms of family 4 in the endoplasmic reticulum. And peroxisome proliferators insignificantly stimulate the expression of ROS detoxification enzymes catalase and glutathione peroxidase [20]. Thus, a disproportion in changes of expression of enzymes responsible for synthesis and degradation of ROS leads to oxidative stress.

NADPH oxidase. NADPH oxidase located in the outer cell membrane is another important source of ROS production. In immune defense cells this enzymatic complex generates ROS capable of killing bacteria. NADPH oxidase is expressed in virtually all cells of animals. During proliferation the cells use ROS produced during functioning of NADPH oxidase as secondary messengers [124]. ROS produced during functioning of NADPH oxidase seem to be involved in carcinogenesis caused by inflammation. No reports have been found explaining carcinogenic effect of any substance by activation of NADPH oxidase. However, the activity of NADPH in tumors is often higher than in normal homologous tissues

[125]. This might be associated with an enhanced proliferative status of tumor cells compared to normal cells.

The literature data presented indicate that ROS generated in the cell under the influence of chemical compounds can create conditions favorable for tumor promotion. These conditions are: uncontrolled cell growth, inhibition of apoptosis and activity of protein p53, changes in cell morphology specific for invasion and metastasizing, etc. The mechanism of ROS action on the cell functions is diverse and not quite clear. However, the ability of ROS to induce pseudohypoxia and as a result create an increased level of HIF-1α in the cell seems to be an important component of tumorigenicity of non-genotoxic substances. This is confirmed by hereditary tumors associated with mutations in the genes encoding VHL, succinate dehydrogenase, or fumarase, which also increase the level of HIF-1α in the state of normoxia.

Acting as a promoter and inducing pseudohypoxia, ROS are likely to be responsible for the oncogenic potential of previously spontaneously initiated cells. But it is also possible that constant ROS-induced stimulation of proliferation and inhibition of apoptosis and protein p53 activity can cause genome instability and oncogenic irreparable mutations leading to generation of initiated cells. Thus, oncogenic mutations can arise as an indirect result of the action of ROS. This does not exclude the generation of initiated cells at higher mutagenic concentrations of ROS due to their direct action.

Under the influence of chemical substances, ROS can be produced by different mechanisms depending on structure of the substance.

Most reasonably, ROS production seems to be associated with oxidation of carcinogens by cytochrome *P450* isoforms because the overwhelming majority of environmental carcinogens are substrates of enzymes of this family. However, the data considered in the present review indicate that ROS can also be produced in mitochondria under the influence of Ah receptor ligands, which include such compounds as chlorinated hydrocarbons and PAH. But it is unclear how the activated Ah receptor can influence functions of mitochondria.

Generation of ROS as a promoting factor during chemical carcinogenesis suggests that antioxidants can act as anti-carcinogens during the promotion stage of tumor development. But because ROS can be generated in different cell compartments through different mechanisms depending on the agent, it seems that action of antioxidants should be coupled with generation of ROS. Thus, in the case of ROS production in mitochondria the action of antioxidants must be directed to these organelles and in the case of cytochrome *P450*-dependent production of ROS antioxidants must act within the endoplasmic reticulum.

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REFERENCES

1. Kobliakov, V. A. (1998) *Biochemistry (Moscow)*, **63**, 885-898.
2. Peters, J. M., Cattley, R. C., and Gonzalez, F. J. (1997) *Carcinogenesis*, **18**, 2029-2033.
3. Yamamoto, Y., Moore, R., Goldsworthy, T. L., Negishi, M., and Maronpot, R. R. (2004) *Cancer Res.*, **64**, 7197-7200.
4. Courtney, G. H., Ho, A., Yoshioka, N., and Dowdy, S. F. (2006) *Mol. Cell. Biol.*, **26**, 4701-4711.
5. Menon, S., Sarsour, E., Spitz, D., Higashikuba, R., Sturm, M., Zhang, H., and Gaswami, P. (2003) *Cancer Res.*, **63**, 2109-2117.
6. Wu, W. S. (2006) *Cancer Metast. Rev.*, **25**, 695-705.
7. Adler, V., Zhimin, Y., Tew, K. D., and Ronai, Z. (1999) *Oncogene*, **18**, 6104-6111.
8. Adler, V., Yin, Z., Fuchs, S. Y., Benezra, M., Rosario, L., Tew, K. D., Pincus, M. R., Sardana, M., Henderson, C. J., Wolf, C. R., and Ronai, Z. (1999) *EMBO J.*, **18**, 1321-1334.
9. Villafania, A., Anwar, K., Amar, S., Chie, L., Way, D., Chung, D. L., Adler, V., and Ronai, Z. (2000) *Ann. Clin. Lab. Sci.*, **30**, 57-64.
10. Giles, G. (2006) *Curr. Pharm. Design*, **12**, 4427-4443.
11. Fujiki, H., and Suganuma, M. (2009) *Prog. Mol. Subcell. Biol.*, **46**, 221-254.
12. Slaga, T. J., Klein-Szanto, A. J., Triplett, L. L., Yotti, L. P., and Trosko, K. E. (1981) *Science*, **213**, 1023-1025.
13. Rohr-Udilova, N. V., Stolze, K., Sagmeister, S., Nohl, H., Schulte-Hermann, R., and Grasl-Kraupp, B. (2008) *Mol. Nutr. Food Res.*, **52**, 352-359.
14. Giri, U., Iqbal, M., and Athar, M. (1996) *Carcinogenesis*, **17**, 2023-2028.
15. Teufelhofer, O., Parzefall, W., Kainzbauer, E., Ferk, F., Freiler, C., Knasmuller, S., Elbling, L., Thurman, R., and Schulte-Herman, R. (2005) *Carcinogenesis*, **26**, 319-329.
16. Peters, J. M., and Gonzalez, F. (2005) *J. Mol. Med.*, **83**, 774-785.
17. Ohshima, H., Tatemichi, M., and Sawa, T. (2003) *Arch. Biochem. Biophys.*, **417**, 3-11.
18. Keisari, Y., Flescher, E., and Geva, I. (1984) *Int. J. Cancer*, **34**, 845-848.
19. Cerutti, P. A. (1985) *Science*, **227**, 375-381.
20. Yeldandi, A. V., Rao, M. S., and Reddy, J. K. (2000) *Mutat. Res.*, **448**, 159-177.
21. Alsharif, N. Z., Lawson, T., and Stohs, S. J. (1994) *Toxicology*, **92**, 39-51.
22. Dostalek, M., Brooks, J. D., Hardy, K. D., Milne, G. L., Moore, M. M., Sharma, S., Morrow, J. D., and Guengerich, F. R. (2007) *Mol. Pharmacol.*, **72**, 1419-1424.
23. Park, J. Y., Shigenaga, M. K., and Ames, B. N. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 2322-2327.
24. Schulte-Hermann, R., Timmermann-Trosiener, I., Barthel, G., and Bursch, W. (1990) *Cancer Res.*, **50**, 5127-5135.
25. Stohs, S. J., Alsharif, N. Z., Shara, M. A., Al-Bayati, Z. A., and Wahba, Z. Z. (1991) *Adv. Exp. Med. Biol.*, **283**, 827-831.

26. Gottlieb, E., and Tomlinson, I. P. (2005) *Nat. Rev. Cancer*, **5**, 857-866.
27. Lee, K., Roth, R., and LaPres, J. (2007) *Pharmacol. Therap.*, **113**, 229-246.
28. Chandel, N. S., and Budinger, G. R. (2007) *Free Rad. Biol. Med.*, **42**, 165-174.
29. Piret, J. P., Minet, E., Cosse, J. P., Ninane, N., Debacq, C., Raes, M., and Michiels, C. (2005) *J. Biol. Chem.*, **280**, 9336-9644.
30. King, A., Selak, M. A., and Gottlieb, E. (2006) *Oncogene*, **25**, 4675-4682.
31. Koivunen, P., Hirsil, M., Remes, A. M., Hassinen, I. E., Kivirikko, K. I., and Myllyharju, J. (2007) *J. Biol. Chem.*, **282**, 4524-4532.
32. Chandel, N. S., McClintock, D. S., Feliciano, C. E., Wood, T. M., Melendez, J. A., Rodriguez, A. M., and Schumaker, R. T. (2000) *J. Biol. Chem.*, **275**, 25130-25138.
33. Jung, S. N., Yang, W. K., Kim, J., Kim, H. S., Kim, E. J., Yun, H., Park, H., Kim, S. S., Choe, W., Kang, I., and Ha, J. (2008) *Carcinogenesis*, **29**, 713-721.
34. Mansfield, K. D., Guzy, R. D., Pan, Y., Young, R. M., Cash, T. P., Schumaker, P. T., and Simon, M. C. (2005) *Cell Metab.*, **1**, 393-399.
35. Kohl, R., Zhou, J., and Brune, B. (2006) *Free Rad. Biol. Med.*, **40**, 1430-1442.
36. Haddad, J. J. (2002) *Eur. Cytokine Netw.*, **13**, 250-260.
37. Park, J. H., Kim, T. Y., Jong, H. S., Kim, T. Y., Chun, Y. S., Park, J. W., Lee, C. T., Jung, H. C., Kim, N. K., and Bang, Y. J. (2003) *Clin. Cancer Res.*, **9**, 433-440.
38. Yang, H., and Kaelin, W. G., Jr. (2001) *Cell Growth Differentiation*, **12**, 447-455.
39. Pollard, P. J., Briere, J. J., Alam, N. A., Barwell, J., Barclay, E., Wortham, N. C., Hunt, T., Mitchell, M., Olpin, S., Moat, S. J., Hargreaves, I. P., Heales, S. J., Chung, Y. L., Griffiths, J. R., Dalgleish, A., McGrath, J. A., Gleeson, M. J., Hodgson, S. V., Poulsom, R., Rustin, P., and Tomlinson, I. P. (2005) *Hum. Mol. Genet.*, **14**, 2231-2239.
40. Baysal, B. E. (2006) *Endocr. Pathol.*, **17**, 203-212.
41. Chen, H., and Costa, M. (2006) *Exp. Biol. Med. (Maywood)*, **231**, 1474-1480.
42. Ahn, Y. S., Chemeris, G. Y., Turusov, V. S., and Bannasch, P. (1994) *Toxicol. Pathol.*, **22**, 415-422.
43. Arulkumaran, S., Ramprasath, V. R., Shanthi, P., and Sachdanandam, P. (2007) *Chem. Biol. Interact.*, **167**, 99-106.
44. Zhao, T., Zhang, C. P., Liu, Z. H., Wu, L. Y., Huang, X., Wu, H. T., Xiong, L., Wang, X., Wang, X. M., Zhu, L. L., and Fan, M. (2008) *FEBS J.*, **275**, 1824-1834.
45. Lee, S. H., Lee, M. Y., and Han, H. J. (2008) *Cell Prolif.*, **41**, 230-247.
46. Di Carlo, A., De Mori, R., Martelli, F., Pompilio, G., Capogrossi, M. C., and Germani, A. (2004) *J. Biol. Chem.*, **279**, 16332-16338.
47. Lin, Q., Lee, Y. J., and Yun, Z. (2006) *J. Biol. Chem.*, **281**, 30678-30683.
48. Cowden, K. D., Robertson, S. E., Weaver, V. M., and Simon, M. C. (2005) *Mol. Biol. Cell*, **16**, 1901-1912.
49. Krishnamachary, B., Berg-Dixon, S., Kelly, B., Agani, F., Feldser, D., and Ferreira, G. (2003) *Cancer Res.*, **63**, 1138-1143.
50. Piret, J. P., Minet, E., Cosse, J. P., Ninane, N., Debacq, C., Raes, M., and Michiels, C. (2005) *J. Biol. Chem.*, **280**, 9336-9644.
51. Brahimi-Horn, M. C. (2007) *FEBS Lett.*, **581**, 3582-3591.
52. Gwak, G. Y., Yoon, J. H., Kim, K. M., Lee, H. S., Chung, J. W., and Gores, G. J. (2005) *J. Hepatol.*, **42**, 358-364.
53. Lu, C. W., Lin, S. C., Chen, K. F., Lai, Y. Y., and Tsai, S. J. (2008) *J. Biol. Chem.*, **283**, 28106-28114.
54. Minamino, T., Mitsialis, S. A., and Kourembanas, S. (2001) *Mol. Cell Biol.*, **21**, 3336-3342.
55. Yatabe, N., Kyo, S., Maida, Y., Nishi, H., Nakamura, M., Kanaya, T., Tanaka, T., Tanaka, M., Isaka, K., Ogawa, S., and Inoue, M. (2004) *Oncogene*, **23**, 3708-3715.
56. Nishi, H., Nakada, T., Kyo, S., Inoue, M., Shay, J. W., and Isaka, K. (2004) *Mol. Cell Biol.*, **24**, 6076-6083.
57. Anderson, C. J., Hoare, S. F., Ashcroft, M., Bilsland, A. E., and Keith, W. N. (2006) *Oncogene*, **25**, 61-69.
58. Phesse, T. J., and Clarke, A. R. (2009) *Br. J. Cancer*, **100**, 221-227.
59. Wicha, M. S., Liu, S., and Dontu, G. (2006) *Cancer Res.*, **66**, 1883-1890.
60. Simon, M. C., and Keith, B. (2008) *Nat. Rev. Mol. Cell Biol.*, **9**, 285-296.
61. Keith, B., and Simon, M. C. (2007) *Cell*, **129**, 465-472.
62. Cipolleschi, M. G., Dello Sbarba, P., and Olivotto, M. (1993) *Blood*, **82**, 2031-2037.
63. Covello, K. L., Kehler, J., Yu, H., Arsham, A. M., Hu, C. J., Labosky, P. A., Simon, M., and Keith, B. (2006) *Genes Dev.*, **20**, 557-570.
64. Gustafsson, M. V., Zheng, X., Pereira, T., Gradin, K., Jin, S., Lundkwist, J., Ruas, J. L., Poelinger, L., Lendahl, U., and Bondesson, M. (2005) *Dev. Cell*, **9**, 617-628.
65. Diez, H., Fischer, A., Winkler, A., Hu, C. J., Hatzopoulos, A. K., Breier, G., and Gessler, M. (2007) *Exp. Cell Res.*, **313**, 1-9.
66. Poellinger, L., and Lendahl, U. (2008) *Curr. Opin. Genet. Dev.*, **18**, 449-454.
66a. Sahlgren, C., Gustafsson, M., Jin, S., Poellinger, L., and Lendahl, U. (2008) *Proc. Natl. Acad. Sci. USA*, **105**, 6392-6397.
67. Wilson, A., and Radtke, F. (2006) *FEBS Lett.*, **580**, 2860-2868.
68. Comerford, K. M., Wallace, T. J., Karhausen, J., Louis, N. A., Montalto, M. C., and Sean, P. (2002) *Cancer Res.*, **62**, 3387-3394.
69. Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., and Mazur, M. (2006) *Chem. Biol. Interact.*, **160**, 1-40.
70. Galanis, A., Pappa, A., Giannakakis, A., Lanitis, E., Dangaj, D., and Sandaltzopoulos, R. (2008) *Cancer Lett.*, **266**, 12-20.
71. Schmid, T., Zhou, J., and Brune, B. (2004) *J. Cell Mol. Med.*, **8**, 423-431.
72. Peng, X. H., Karna, P., Cao, Z., Jiang, B. H., Zhou, M., and Yang, L. (2006) *J. Biol. Chem.*, **281**, 25903-25914.
73. Yang, L., Cao, Z., Li, F., Post, D. E., van Meir, E. G., Zhong, H., and Wood, W. C. (2004) *Gene Ther.*, **11**, 1215-1223.
74. Villafania, A., Anwar, K., Amar, S., Chie, L., Way, D., Chung, D. L., Adler, V., and Ronai, Z. (2000) *Ann. Clin. Lab. Sci.*, **30**, 57-64.
75. Duffy, M. J., O'Donovan, N., Brennan, D. J., Gallagher, W. M., and Ryan, B. M. (2007) *Cancer Lett.*, **249**, 49-60.
76. Hsue, S. S., Wang, W. C., Chen, Y. K., and Lin, L. M. (2008) *Int. J. Exp. Pathol.*, **89**, 309-320.

77. Abu-Hamad, S., Zaid, H., Israelson, A., Nahon, E., and Shoshan-Barmatz, V. (2008) *J. Biol. Chem.*, **283**, 13482-13490.
78. Vyssokikh, M., and Brdiczka, D. (2004) *Mol. Cell Biochem.*, **256/257**, 117-126.
79. Pastorino, J. G., and Hoek, J. B. (2003) *Curr. Med. Chem.*, **10**, 1535-1551.
80. Sun, L., Shukair, S., Naik, T., Moazed, F., and Ardehali, H. (2008) *Mol. Cell. Biol.*, **28**, 1007-1017.
81. Abu-Hamad, S., Arbel, N., Calo, D., Arzoine, L., Israelson, A., Keinan, N., Ben-Romano, R., Friedman, O., and Shoshan-Barmatz, V. (2009) *J. Cell. Sci.*, **122**, 1906-1916.
82. Li, J., Zhang, X., Sejas, D. P., Bagby, G., and Pang, Q. (2004) *J. Biol. Chem.*, **279**, 41275-41279.
83. Maiguel, D. A., Jones, L., Chakravarty, D., Yang, C., and Carrier, F. (2004) *Mol. Cell. Biol.*, **24**, 3703-3711.
84. Li, J., Zhang, X., Sejas, D. P., and Pang, Q. (2005) *Leuk. Res.*, **29**, 1415-1423.
85. Piret, J. P., Minet, E., Cosse, J. P., Ninane, N., Debacq, C., Raes, M., and Michiels, C. (2005) *J. Biol. Chem.*, **280**, 9336-9644.
86. Radisky, D. C. (2005) *J. Cell Sci.*, **118**, 4325-4326.
87. Cannito, S., Novo, E., Compagnone, A., Valfri di Bonzo, L., Busletta, C., Zamara, E., Paternostro, C., Povero, D., Bandino, A., Bozzo, F., Cravanzola, C., Bravoco, V., Colombatto, S., and Parola, M. (2008) *Carcinogenesis*, **29**, 2267-2278.
88. Imai, T., Horiuchi, A., Wang, C., Oka, K., Ohira, S., Nikaido, T., and Konishi, I. (2003) *Am. J. Pathol.*, **163**, 1437-1447.
89. Russell, R. C., and Ohh, M. (2007) *Cell Cycle*, **6**, 56-59.
90. Leong, K. G., Niessen, K., Kulic, I., Raouf, A., Eaves, C., Pollet, I., and Karsan, A. (2007) *J. Exp. Med.*, **204**, 2935-2948.
91. Peinado, H., Carmen Iglesias-de la Cruz, M., Olmeda, D., Csiszar, K., Fong, K., Vega, S., Nieto, M., Cano, A., and Portillo, F. (2005) *EMBO J.*, **24**, 3446-3458.
92. Huber, M. A., Kraut, N., and Beug, H. (2005) *Curr. Opin. Cell Biol.*, **17**, 548-558.
93. Zavadil, J., and Boettinger, E. P. (2005) *Oncogene*, **24**, 5764-5774.
94. Yang, M. H., and Wu, K. J. (2008) *Cell Cycle*, **7**, 2090-2096.
95. Yang, M. H., Wu, M. Z., Chiou, S. H., Chen, P. M., Chang, S. Y., Liu, C. J., Teng, S. C., and Wu, K. J. (2008) *Nat. Cell Biol.*, **10**, 295-305.
96. Baghi, M., and Stohs, S. J. (1993) *Free Rad. Biol. Med.*, **14**, 11-18.
97. Shertzer, H. G., Clay, C. D., Genter, M. B., Chames, M. C., Schneider, S. N., Oakley, G. G., Nebert, D. W., and Dalton, T. P. (2004) *Free Rad. Biol. Med.*, **36**, 618-631.
98. Schlezinger, J. J., Struntz, W. D., Goldstone, J. V., and Stegeman, J. J. (2006) *Aquat. Toxicol.*, **77**, 422-432.
99. Dostalek, M., Brooks, J. D., Hardy, K. D., Milne, G. L., Moore, M. M., Sharma, S., Morrow, J. D., and Guengerich, F. P. (2007) *Mol. Pharmacol.*, **72**, 1419-1424.
100. Shertzer, H. G., Genter, M. B., Shen, D., Nebert, D. W., Chen, Y., and Dalton, T. P. (2006) *Toxicol. Appl. Pharmacol.*, **217**, 363-374.
101. Howell, A. N., and Sager, R. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 2358-2362.
102. Giguere, L., and Morais, R. (1981) *Somatic Cell Genet.*, **7**, 457-471.
103. Jonasson, J., Povey, S., and Harris, H. (1977) *J. Cell Sci.*, **24**, 217-254.
104. Biswas, G., Guha, M., and Avadhani, N. G. (2005) *Gene*, **354**, 132-139.
105. Amuthan, G., Biswas, G., Zhang, S. Y., Klein-Szanto, A., Vijayasarathy, C., and Avadhani, N. G. (2001) *EMBO J.*, **20**, 1910-1920.
106. Biswas, G., Anandatheerthavarada, H. K., and Avadhani, N. G. (2005) *Cell Death Differ.*, **12**, 266-278.
107. Baghi, M., and Stohs, S. J. (1993) *Free Rad. Biol. Med.*, **14**, 11-18.
108. Stohs, S. J., Shara, M. A., Alsharif, N. Z., Wahba, Z. Z., and Al-Bayati, Z. A. (1990) *Toxicol. Appl. Pharmacol.*, **106**, 126-135.
109. Alsharif, N. Z., Grandjean, C. J., Murray, W. J., and Stohs, S. J. (1990) *Xenobiotica*, **20**, 979-988.
110. Bagchi, M., Bagchi, D., and Stohs, S. J. (1996) *Arch. Environ. Contam. Toxicol.*, **30**, 418-422.
111. Senft, A., Dalton, T. P., Nebert, D. W., Genter, M. B., Hutchinson, R. J., and Shertzer, H. G. (2002) *Toxicol. Appl. Pharmacol.*, **178**, 15-21.
112. Chandel, N. S., and Budinger, G. R. (2007) *Free Rad. Biol. Med.*, **42**, 165-174.
113. Elchuri, S., Oberley, T. D., Qi, W., Eisenstein, R. S., Jackson, R. L., van Remmen, H., Epstein, C. H., and Huang, T. T. (2005) *Oncogene*, **24**, 367-380.
114. Zhao, Y., Chaiswing, L., Oberley, T. D., Batinic-Haberle, I., and St. Clair, W. (2005) *Cancer Res.*, **65**, 1401-1405.
115. Oberley, T. D., Xue, Y., Zhao, Y., Kiningham, K., Szweda, L. I., and St. Clair, D. K. (2004) *Antioxid. Redox Signal.*, **6**, 537-548.
116. Genter, M. B., Clay, C. D., Dalton, T. P., Dong, H., Nebert, D. W., and Shertzer, H. G. (2006) *Biochem. Biophys. Res. Commun.*, **342**, 1375-1381.
117. Senft, A., Dalton, T. P., Nebert, D. W., Genter, M. B., Puga, A., Hutchinson, R. J., Kerzee, J. K., Uno, S., and Shertzer, H. G. (2002) *Free Rad. Biol. Med.*, **33**, 1268-1278.
118. Moennikes, O., Loeppen, S., Buchmann, A., Andersson, P., Ittrich, C., Poellinger, L., and Schwarz, M. (2004) *Cancer Res.*, **64**, 4707-4710.
119. Andersson, P., Rubio, C., Poellinger, L., and Hanberg, A. (2005) *Anticancer Res.*, **25**, 903-911.
120. Traore, K., Sharma, R., Thimmulappa, R. K., Watson, W. H., Biswal, S., and Trush, M. A. (2008) *J. Cell. Physiol.*, **216**, 276-285.
121. Wang, Y., Biswas, G., Prabu, S. K, and Avadhani, N. G. (2006) *Biochem. Pharmacol.*, **72**, 881-892.
122. Fisher, M. T., Nagarkatti, M., and Nagarkatti, P. S. (2005) *Toxicol. Lett.*, **157**, 99-107.
123. Shen, D., Dalton, T. P., Nebert, D. W., and Shertzer, H. G. (2005) *J. Biol. Chem.*, **280**, 25305-25312.
124. Elsa, C., Jiang, C., Peshavariya, H. M., and Dusting, G. (2009) *Pharmacol. Therap.*, **122**, 97-108.
125. Lambeth, D. J. (2007) *Free Rad. Biol. Med.*, **43**, 332-347.